IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Patent No.	:	**6,034,267**
Issue Date	:	March 7, 2000
Applicant	:	Gierskcky et al.
Title	:	ESTERS OF 5-AMINOLEVULINIC ACID AS PHOTOSENTIZING AGENTS IN PHOTOCHEMOTHERAPY

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DECLARATION OF DR. KRISTIAN BERG IN SUPPORT OF GRANT OF PATENT TERM EXTENSION WITH RESPECT TO U.S. PATENT NO. 6,034,267

I, Dr. Kristian Berg, of Oslo, Norway, hereby declare:

1. I have held the position of senior scientist at The Norwegian Radium Hospital, Oslo Norway, since 1997.[1] Prior to that, I held the position of scientist at The Norwegian Radium Hospital from 1985 to 1997. My resume is attached as Exhibit 1.

2. I have read and understand United States Patent No. 6,034,267 ("the '267 patent," Exhibit 2), which discloses and claims a method of diagnosing or treating a variety of disorders and abnormalities by administering esters of aminolevulinic acid, particularly including the methyl ester and its salts such as the hydrochloride salt, and then exposing the disorder or abnormality to light. I provided a Declaration [Exhibit 3] that was submitted during the prosecution of United States Patent Application No. 08/913,257, which issued as United States Patent No. 6,034,267. I have also read and understand the references cited in the Berg Declaration.

3. Photodynamic therapy is a technique developed for the treatment of various abnormalities or disorders of the skin or other epithelial organs or mucosa, especially cancers or pre-cancerous lesions, as well as certain non-malignant lesions, including skin complaints such as psoriasis. Photodynamic therapy involves the application of photosensitizing agents to the affected area of the body, followed by exposure to photoactivating light in order to activate the photosensitizing agents and convert them into cytotoxic form, whereby the affected cells are killed or their proliferative potential diminished.

4. I have substantial experience in photodynamic therapy ("PDT"), having worked in this area for many years. In particular, I have conducted, designed, and supervised experimental work with 5-aminolevulinic acid (ALA) and esters of ALA (including their salts) in photodynamic therapy

[1] The NRH is the national cancer hospital for Norway and it is the largest comprehensive cancer centre in Northern Europe. The NRH is part of the medical school of the University of Oslo and includes a clinical hospital as well as the Institute of Cancer Research.

since 1991 and have investigated the differences in effect of ALA and ALA esters when used in PDT and also the mechanisms of action of ALA and ALA esters in PDT. I have also written and published several reviews on this topic in peer-reviewed journals, and I have chaired symposia related to the use of ALA and ALA-esters in PDT.

5. A range of photosensitizing agents have been investigated, among the most important of which are the porphyrins. However, porphyrin-based drugs have a number of disadvantages including the fact that they do not readily penetrate the skin when applied topically. 5-Aminolevulinic acid ("ALA") was identified as a potential alternative to porphyrin-based drugs. ALA is a natural precursor of protoporphyrin IX ("PpIX"), a naturally-occurring product that has photosensitizing activity. ALA is formed from succinyl CoA and glycine in the first step of heme synthesis. ALA was investigated as a PDT agent for certain skin cancers. It is able to penetrate the skin to a limited extent and its topical application leads to a localized build-up of PpIX. Thus, by applying ALA topically to skin tumours, and then after several hours exposing the tumours to light, a beneficial PDT effect may be obtained.

6. I am informed that the United States Patent and Trademark Office has rejected a petition for patent term extension for United States Patent No. 6,034,267 because the corresponding product, Metvixia™, has as its active ingredient the hydrochloride salt of the methyl ester of ALA (methyl aminolevulinate hydrochloride), and therefore is considered the same as the previously marketed product Levulan®, which has the hydrochloride salt of ALA as its active ingredient.

7. However, photochemotherapy with ALA is not always entirely satisfactory. ALA is not able to penetrate all tumours and other tissues with sufficient efficacy to enable treatment of a wide range of tumours or other conditions. The '267 patent inventors investigated whether ALA esters, including methyl aminolevulinate hydrochloride, could provide clinical benefits superior to those of ALA. Additional studies were later performed to further characterize the identified differences under a variety of conditions. These studies and others, performed both *in vitro* and *in vivo* by or under the supervision of the inventors or as otherwise detailed below, identified substantial differences between ALA and esters of ALA, including the hydrochloride salts, as set forth in the '267 patent, the Berg Declaration, or as otherwise set forth below.[2]

8. More particularly, substantial differences between methyl aminolevulinate hydrochloride and ALA hydrochloride include the following:

selectivity of uptake by target lesions,

penetration of target lesions,

(unwanted) systemic distribution of the active ingredient,

pain resulting from use in PDT, and

mechanisms by which cells take up the active ingredient.

In light of these substantial differences, it is my opinion that ALA hydrochloride and methyl aminolevulinate hydrochloride should not be considered the same active ingredient.

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[2] It is my understanding that all properties of ALA and methyl aminolevulinate set forth below were observed and measured in experiments that used the hydrochloride (HCl) salt of the corresponding compound, such as ALA HCl or methyl aminolevulinate HCl.

9. **Selectivity of Uptake by Target Lesions**: ALA esters such as methyl aminolevulinate hydrochloride differ from ALA in that ALA esters display a significantly increased selectivity for tumour tissue versus surrounding non-tumour tissue, as research on ALA esters shows. Exemplary references and data are set forth below. *See also* '267 patent at col. 14, ll. 45-60 and Figures 4-7, col. 15, ll. 23-42 and Figures 8-11, and col. 16, ll. 11-27 and Figures 14-17, and Berg Declaration at ¶ 14. This property of the ALA-ester is clearly advantageous for PDT.

9(a). Exhibit 4 contains two photographs of the same lesion on a patient's arm to which Metvix (the original name for the formulation now known as Metvixia™) was applied and left for 3 hours before being removed. Dr. Warloe, an inventor of the '267 patent, performed this work. Dr. Warloe took these photographs at the NRH and subsequently provided them to PhotoCure. The photograph on the left shows a shoulder under blue light and the red fluorescence of the photoactive porphyrins in the lesion. The photograph on the right was taken before Metvix was applied and shows the same lesion in normal light where the lesion is clearly visible to the naked eye. The lesion visible in the photograph on the right matches the red fluorescence in the photograph on the left. This photograph demonstrates the selectivity of Metvix for abnormal cells over normal skin.

9(b). Exhibit 5 contains two photographs of a patient's arm. In the photograph on the left, Metvix and ALA in a cream formulation have each been applied to normal skin on the patient's arm. The area where ALA was applied has a substantial amount of fluorescence whereas the area where Metvix was applied has minimal fluorescence. The photograph on the right shows a lesion where Metvix was applied to one half and ALA to the other. Both creams were applied to the lesion and the area surrounding the lesion. The Metvix half has discrete patches of fluorescence corresponding to the lesion whereas ALA has caused fluorescence in both the lesion and the surrounding normal skin. It can be concluded that Metvix is selective for abnormal skin whereas ALA is not. These photographs were provided to PhotoCure by Professor Wulf from the Department of Dermatology, University of Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark, who conducted this experiment on three patients independently of PhotoCure. Professor Wulf obtained similar results for all three patients.

9(c). Exhibit 6 contains a graph which shows the ratio of fluorescence present in basal cell carcinoma cells relative to the fluorescence in normal surrounding skin cells after application of methyl aminolevulinate. A high fluorescence ratio means that there is high tumour selectivity. The figure shows that tumour selectivity is at its highest a few hours after cream application (about 20 times as much fluorescence in the lesion as in normal skin), decreasing to about 8 times as much fluorescence 28 hours after application. The fluorescence ratio is shown on a log scale. This graph was prepared by Even Angell-Petersen as part of his work at the NRH under Dr. Warloe's supervision during Photocure's Phase II trial called PCT-206. Mr. (now Dr.) Angell-Petersen provided this graph to PhotoCure and presented it at the European Academy of Dermatology and Venerology, 15-18 October 2003. An abstract of this presentation was published in J. Eur. Acad. of Dermatol. and Venerol. 17 (suppl. 3): 126 (November 2003).

9(d). Exhibit 7 shows a graph similar to the previous exhibit except that here actinic keratoses cells are compared to normal cells after application of methyl aminolevulinate. For actinic keratoses, lesion selectivity ranges from 20 to 5 times as much fluorescence in the lesion as in normal skin, depending on application time. This graph was prepared by Even Angell-Petersen as part of his work at the NRH under Dr. Warloe's supervision during PhotoCure's Phase II trial called PCT-206. Mr. (now Dr.) Angell-Petersen provided the graph to PhotoCure and presented it at the European Academy of Dermatology and Venerology 15- 18 October 2003. An abstract of

this presentation was published in J. Eur. Acad. of Dermatol. and Venerol. 17 (suppl. 3): 126 (November 2003).

9(e). Exhibit 8, Fritsch et al. (1998), studies the porphyrin fluorescence induced in actinic keratosis skin lesions and the skin surrounding the lesion. The article concludes that the ratio of porphyrin fluorescence in the lesion when compared to normal skin was higher for methyl aminolevulinate than ALA. This indicates that the methyl aminolevulinate had greater selectivity for lesional skin over normal skin than did ALA.

9(f). Exhibit 9, Thompson et al. (2001), studies the porphyrin fluorescence induced in basal cell carcinoma skin lesions and the skin surrounding the lesion. The article concludes that the porphyrin fluorescence builds up more selectively in the lesions following use of methyl aminolevulinate than use of ALA.

9(g). Exhibit 10, Wiegell and Wulf (2006), report that it was observed in patients treated for acne that ALA induced a homogeneous accumulation of PpIX whereas PpIX induced by methyl aminolevulinate was distributed in spots, indicating that methyl aminolevulinate was more selective for acne lesions over neighboring normal skin than was ALA.

9(h). Exhibit 11, Peng et al. (2001), reports that after application of Metvix (same formulation as Metvixia™) to basal cell carcinoma and surrounding normal skin for a period of three hours or eighteen hours, fluorescence was induced "highly selectively" in the lesions, with "no significant methyl 5-aminolevulinate-induced porphyrin fluroescence in normal dermis." Peng et al., 142-43.

10. **Penetration of Target Lesions**: Methyl aminolevulinate is able to penetrate better into thick basal cell carcinoma lesions than ALA. Further, the penetration is not only deeper but more evenly distributed in the lesion leading to more efficient treatment. ALA penetrates such thick lesions only at the surface and in an irregular patchy fashion leading to incomplete treatment of the lesion. The data set forth below illustrates this phenomenon. *See also* '267 patent at col. 15, ll. 23-31 and Figures 8-9 and Berg Declaration at ¶ 16.

10(a). Exhibit 12 is a graph illustrating the penetration of methyl aminolevulinate (P-1202 represented by the black dots) in comparison to the penetration of ALA (represented by white dots). Dr. Peng, an inventor of the '267 patent, prepared this graph. He subsequently provided a copy to PhotoCure. Dr. Peng collected the data for ALA as part of his research at the NRH. Dr. Peng collected the data for methyl aminolevulinate as part of his work at the NRH during PhotoCure's first Phase I trial called PCT-101. Methyl aminolevulinate is shown to penetrate to 2 mm whereas ALA penetrates less than 1 mm into the lesion. This is very important in the treatment of basal cell carcinomas because the lesions are often more than 1 mm thick. Further, the amount of fluorescence induced by methyl aminolevulinate is greater than that for ALA at the same depth.

10(b). Exhibit 13 contains black and white photographs from a biopsy of a basal cell carcinoma lesion to which Metvix has been applied. Dr. Peng took these photographs and prepared the graph as part of his work at the NRH during PhotoCure's first Phase I trial called PCT-101. The image on the left shows the fluorescence of photoactive porphyrins (white) in a BCC lesion 3 hours after application of Metvix. The image on the right is a picture of the same part of the lesion examined by the standard staining used in histology examination (H-E staining) so that the parts with the denser colour represent the lesion cells. A comparison of these two images demonstrates the correlation of the fluorescence within the lesions' margins, and the even

distribution of fluorescence throughout the whole lesion. In addition, the fluorescence is within the lesion boundaries and does not extend into the underlying normal tissue. The graph underneath shows the fluorescence intensities at different depths of the lesion demonstrating that there is fluorescence at depths of more than 1 mm.

10(c). Peng et al. (2001) (Exhibit 11), reports a clinical trial using methyl aminolevulinate on patients with basal cell carcinoma lesions including the work contained in the previous Figure. The article reports that the distribution of porphyrin fluorescence in the lesions was homogeneous with a high ratio of fluorescence depth to tumour depth.

11. **Systemic Distribution of the Active Ingredient:** Unlike ALA, ALA esters are not transported in the blood to other tissues. That is, while ALA undergoes systemic distribution, ALA esters remain localized at the area of topical application. Experiments demonstrating this were carried out by Professor J. Moan, an inventor of the '267 patent, and others under his direction and/or supervision, at the Department of Biophysics, The Norwegian Radium Hospital ("the NRH"), Institute for Cancer Research ("the Institute"). The corresponding data are presented in Exhibits 14-17, where it is shown that topical administration of ALA to a nude mouse resulted in PpIX formation all over the mouse (i.e. a mouse that, under photoactivating light, appears completely red) whereas topical administration of ALA-ester did not result in any PpIX formation outside the application site. Such properties of the ALA-ester are clearly advantageous for PDT. *See also* '267 patent at col. 14, ll. 21-35 and Figure 2 and Berg Declaration at ¶ 13.

11(a). In Exhibit 14 there are three photographs of a nude mouse at different time intervals. Dr. Sørensen, a researcher at the NRH who worked on ALA and methyl aminolevulinate under the supervision of Dr. Moan, took these photographs as part of his work at the NRH on methyl aminolevulinate. He provided a copy of the photographs to PhotoCure. Methyl aminolevulinate was applied to a patch on the side of the mouse and over time the amount of fluorescence increased. Methyl aminolevulinate is thus limited to the site of application and does not spread throughout the body.

11(b). In Exhibit 15, there are three photographs of a nude mouse at different time intervals which correspond to those in the previous figure. Dr. Sørensen took these photographs as part of his work at the NRH on ALA and methyl aminolevulinate. He provided a copy of the photographs to PhotoCure. ALA was applied to a patch on the side of the mouse in the same way as for Figure 14. Over time, not only does the patch to which the ALA was applied fluoresce but the whole mouse fluoresces red. This shows that ALA passes into the circulatory system and spreads throughout the body.

11(c). Exhibit 16 shows the biopsy results from analysing the organs of mice treated with ALA, methyl aminolevulinate ("methyl") (and hexyl aminolevulinate ("hexyl")). These graphs were prepared by PhotoCure based on data provided by Dr. Sørensen and obtained as part of his work on ALA and methyl aminolevulinate at the NRH. The tables show that the mouse treated with ALA had fluorescence on each flank of its body and in its liver by the end of the 24 hour period, whereas there was little, if any, fluorescence in the skin of the opposite flank or the liver of the mice treated with methyl aminolevulinate or hexyl aminolevulinate. This shows that ALA passes into the circulatory system and spreads throughout the body whereas methyl aminolevulinate does not.

11(d). Exhibit 17, Sørensen et al. (1998),[3] reports the work from which Exhibits 14 and 15 were derived. The systemic effect was observed in the mice 6-8 hours after application of the ALA cream, that is, the whole mouse fluoresced under ultraviolet light. Methyl aminolevulinate did not cause fluorescence outside of the site of application, even after 24 hours. The colour versions of the photographs in this article are similar to those in Figures 7 and 8 at 8 hours.

12. **Pain Resulting from Use in PDT**: Another significant distinction that was observed during clinical trials using ALA and ALA esters is that much less severe pain is felt by patients treated with ALA esters than with ALA alone. This unexpected phenomenon may be explained by the fact that ALA but not ALA esters can be transported by the GABA transporter. GABA transporters are located in peripheral neurons, meaning that 5-ALA but not 5-ALA esters should be expected to be preferentially taken up into peripheral nerve endings. *See* Van Oosten et al. (2006) (Exhibit 23). This may thus explain the more severe pain reactions observed during light exposure of basal cell carcinoma lesions after topical application of 5-ALA as compared to 5-ALA methyl ester. This phenomenon is again discussed in Exhibit 10. *See also* Berg Declaration at ¶ 15.

12(a). Exhibit 19, Wiegell et al. (2003), reports a study conducted by doctors in Copenhagen, Denmark into whether the pain caused by ALA and methyl aminolevulinate was different. The study found that ALA did cause significantly more pain than methyl aminolevulinate.

12(b). Exhibit 10, Wiegell and Wulf (2006), reports significantly more pain reported by acne patients twenty-four hours after treatment of acne with ALA PDT than with methyl aminolevulinate PDT.

12(c) Exhibit 20, Moloney and Collins (2007) ("Moloney"), reports a comparison of ALA and methyl aminolevulinate in the treatment of actinic keratosis. In a controlled study of the treatment of actinic keratosis, each of fifteen patients was treated twice, once in each of two lesion areas, one area receiving the local commercial ALA product (Porphin®) and the other area receiving the commercial methyl aminolevulinate product (Metvix™). Metvix™ was applied according to the label; Porphin® was applied so as to maximize production of PpIX. No significant difference in efficacy was observed. *See* Moloney at 89 (Table 1) and 90. Significantly less pain was reported for the methyl aminolevulinate treatment than for the ALA treatment and patients preferred the methyl aminolevulinate treatment to the ALA treatment. *See id.* at 89-90 and Table 2.

13. **Mechanisms by Which Cells Take up the Active Ingredient**: cells appear to take up 5-ALA and ALA esters by different mechanisms.

13(a). 5-ALA is an endogenous delta amino acid with structural similarities to the β-amino acids β-alanine and taurine as well as γ-aminobutyric acid ("GABA") (*see* Exhibit 21, Rud et al. (2000) (Fig. 5) ("Rud")). Moreover, it is known that particular active transport systems exist for amino acids (*see* Rud). Figure 7 of Rud shows the cellular uptake of radiolabelled β-alanine and GABA in the presence of 5-ALA or methylaminolevulinate. The cells were derived from an adenocarcinoma of the colon. It was found that 5-ALA inhibited uptake of β-alanine and GABA, whereas methyl aminolevulinate did not inhibit the uptake of either molecule. The data indicates that, in this cell line, ALA but not methyl aminolevulinate is taken up by cells by mechanisms

[3] This data later was incorporated into the publication Juzeniene et al. (2002), *Topical application of 5-aminolevulinic acid and its methylester, hexylester and octylester derivatives: considerations for dosimetry in mouse skin model*, Photochem Photobiol. 76: 329-34 (Exh. 18).

similar to those that transport β-alanine and GABA. *See also* Berg Declaration at ¶¶ 8-10. Rodriguez et al. have shown in the LM3 mammary adenocarcinoma cell line that wheras ALA is taken up by BETA transporters, none of the studied ALA derivatives, including the methyl aminolevulinate ester, shares the same uptake mechanism. *See* Rodrigues et al. (2006) (Exhibit 24).

13(b). Exhibit 22 (Gederaas et al. (2001)) suggests that uptake of methyl aminolevulinate seems to be due to active transport mechanisms involving transporters of nonpolar amino acids.

14. Taken together these results are evidence that methyl aminolevulinate hydrochloride behaves substantially differently from ALA hydrochloride in PDT and exhibits many advantageous effects and benefits.

15. All statements made herein of my knowledge are true and all statements made herein on information and belief are believed to be true. I understand that the making of false statements in this proceeding is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Dated: 6-11-07

Dr. Kristian Berg

Exhibits

1. Resume

2. United States Patent No. 6,034,267

3. Declaration of Kristian Berg

4. Metvix selectivity (a)

5. Metvix selectivity (b)

6. Selectivity chart

7. AK selectivity chart

8. Fritsch et al. (1998), *Preferential relative porphyrin enrichment in solar keratoses upon topical application of δ-aminolevulinic acid methylester*, Photochem. Photobiol. 68: 218-21.

9. Thompson et al. (2001), *Photodynamic therapy and diagnostic measurements of basal cell carcinomas using esterified and non-esterified δ-aminolevulinic acid*, J. Porphyrins Phthalocyanines 5: 147-153

10. Wiegell and Wulf (2006), *Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid versus methyl aminolevulinate*, J. Am. Acad. Dermatol. 54: 647.

11. Peng et al. (2001), *Selective distribution of porphyrins in skin thick basal cell carcinoma after topical application of methyl 5-aminolevulinate*, J. Photochem. Photobiol. Biol. 62: 140-145.

12. Fluorescence penetration chart

13. BCC Metvix penetration

14. Systemic distribution of methyl aminolevulinate applied to nude mouse

15. Systemic distribution of ALA applied to nude mouse

16. Systemic distribution in mouse of ALA, methyl aminolevulinate, and hexyl aminolevulinate

17. Sørensen et al. (1998), *Formation of protoporphyrin IX in mouse skin after topical application of 5-aminolevulinic acid and its methyl ester*, EUROPTO Conference on Photochemotherapy of Cancer and Other Diseases, Stockholm, Sweden, SPIE 3563: 77-81.

18. Juzeniene et al. (2002), *Topical application of 5-aminolevulinic acid and its methylester, hexylester and octylester derivatives: considerations for dosimetry in mouse skin model*, Photochem Photobiol. 76: 329-34.

19. Wiegell et al. (2003) , *Pain Associated with Photodynamic Therapy Using 5-Aminolevulinic Acid or 5-Aminolevulinic Acid Methylester on Tape-Stripped Normal Skin*, Arch. Dermatol. 139: 1173- 1177.

20. F.J. Moloney and P. Collins (2007), *Randomized, double-blind, prospective study to compare topical 5-aminolaevulinic acid methylester with topical 5-aminolaevulinic acid photodynamic therapy for extensive scalp keratosis*, Brit. J. Dermatol. 157: 87-91.

21. Rud et al. (2000), *5-aminolevulinic acid, but not 5-aminolevulinic acid esters, is transported into adenocarcinoma cells by system BETA transporters*, Photochem Photobiol. 71: 640-7.

22. Gederaas et al. (2001), *Aminolaevulinic Acid Methyl Ester Transport on Amino Acid Carriers in a Human Colon Adenocarcinoma Cell Line*, Photochem Photobiol. 73: 164-9.

23. Van Oosten et al. (2006) *Different pain sensations in photodynamic therapy of nodular basal cell carcinoma Results from a prospective trial and a review of the literature*, Photodiagnosis and Photodynamic Therapy 3: 61-68

24. Rodriguez et al. (2006) *Mechanisms of 5-aminolevulinic acid ester uptake in mammalian cells*, Brit. J. Pharmacol. 147: 825-33.